PAH Biomarkers

- 1-Hydroxypyrene
- 2-Naphthol
- Phenanthrene metabolites
- Benzo[a]pyrene metabolites

F.J.Jongeneelen Ann. Occup. Hyg. 45: 3 (2001); Grimmer et al, Int. Arch. Occup. Environ. Health 69: 231 (1997);Jacob et al, Biomarkers 4: 319 (1999)

Representative Recent Studies on 1-Hydroxypyrene in Urine

Smokers	Nonsmokers	Reference
1.59 nmol/24h	0.784 nmol/24h	Scherer et al., 1999
2.77 nmol/24h	1.0 nmol/24h	Jacob et al., 1999
2.47 nmol/l	1.10 nmol/l	Pastorelli et al., 1999

1-Hydroxypyrene and 2-Naphthol in Urine

	µmol/mol creatinine[a]	
	Smokers	Nonsmokers
1-hydroxypyrene	0.05	0.04
2-naphthol	3.94	1.55

[a] geometric means
H-M Nan et al., Carcinogenesis 22:787 (2001)

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Source: https://www.industrydocuments.ucsf.edu/docs/sgmj0001

PAH Biomarkers

- 1-Hydroxypyrene
- 2-Naphthol
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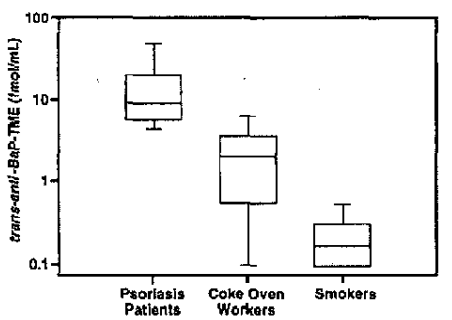
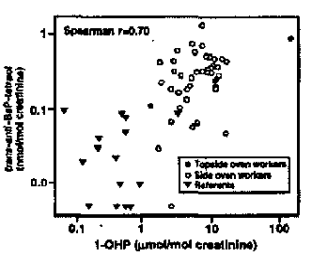
F.J.Jongeneelen Ann. Occup. Hyg. 45: 3 (2001); Grimmer et al, Int. Arch. Occup. Environ. Health 69: 231 (1997); Jacob et al, Biomarkers 4: 319 (1999)

Levels of *trans-anti*-BaP-tetraol in Human Urine



C.D. Simpson et al Chem. Res. Toxicol. 13 271 (2000)

Correlation of *trans-anti*-BaP-tetraol with 1-OHP



M.T. Wu, C. Simpson, D.C. Christiani, L.W. Chang, & S.S. Hecht CEBP, in press (2001)

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Source: https://www.industrydocuments.ucsf.edu/docs/sgmj0001

Aromatic Amine and Heterocyclic Aromatic Amine Biomarkers

- 2-Toluidine: 4.1-6.3 ug/ 24h, no difference between smokers and non-smokers
- 4-Aminobiphenyl: 68-79 ng/ 24h, no difference between smokers and non-smokers
- PhiP: no difference between smokers and non-smokers

Grimmer et al, Sci Tot Env 247: 81 (2000)

Diet accounted for ~~one~~ day.

Benzene Biomarkers

- *trans,trans*-muconic acid (ttMA) has been most frequently used
- 12 of 14 studies showed increased levels of ttMA in smokers
- Ratio: smokers to non-smokers, 1.4-4.8
- Phenylmercapturic acid may be more specific than ttMA

G. Scherer et al, J. Chromatog B, 717: 179 (1998);Proc AACR 42: 150 (2000)

Other Specific Biomarkers

- 3-Hydroxypropyl- and other mercapturic acids (from acrolein, butadiene, ethylene oxide, acrylonitrile)
- Catechol and its conjugates
- 8-Oxodeoxyguanosine and related oxidation products

P.J. Boogard et al, Chem. Biol. Int. 135: 695 (2001); S.G. Carmella et al, Food Chem. Toxicol 20: 587 (1982); G. Scherer et al, Mutation Res. 366: 195 (1996); G Scherer et al., Proc AACR, 42: 148 (2000); D. Mascher et al, J. Chromatog. B 750: 163 (2001); T. Renner et al, J. Chromatog. B 738: 311 (2000)

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Source: https://www.industrydocuments.ucsf.edu/docs/sgmj0001

Non-specific biomarkers

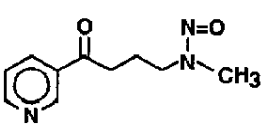
- Thioethers
- Mutagenicity (Diet)
- Alkylmercapturic acids
- Alkyladenines

G. Scherer et al, Mutation Res. 368: 195 (1996);
A. Kopplin et al, Carcinogenesis 16: 2637 (1995)

Nitrosamine Biomarkers

- Nitrosamino Acids
- NNAL and NNAL-Glucs

Reviewed by Mirvish, Cancer Lett 93: 17 (1995)
Carmella et al, Cancer Res. 53: 721 (1993)



NNK (nicotine-derived nitrosamino ketone)

- Present in tobacco and tobacco smoke; tobacco-specific
- Powerful pulmonary carcinogen in rodents
- Induces tumors at total doses similar to human dose
- Similar metabolic activation pathways in rodents and humans

S.S. Hecht, Chem. Res. Toxicol. 11:559-603 (1998)
S.S. Hecht and D. Hoffmann, Carcinogenesis 9:875-884 (1988)

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Source: https://www.industrydocuments.ucsf.edu/docs/sgmj0001

Induction of Lung Tumors by NNK

Species and Strain	Route
Mouse	
A/J	i.p., gavage, p.o.
Sencar	skin
BALB/c	oral
Swiss	oral, i.p.
$C3B6F_1$	i.p.
C3H/HeJ	i.p.
C57BL/6	i.p.
$(A/J \times TSG\text{-}p53)F_1$	i.p.
F-344 Rat	s.c., p.o., oral swab, gavage, intravesicular
Syrian Golden Hamster	s.c., application to cheek pouch
Mink	s.c.

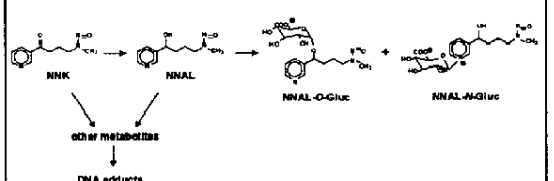
S.S. Hecht, Chem. Res. Toxicol.,11: 559-603, 1998

Relative Risk for Lung Cancer in Cigar Smokers

	Odds Ratio (95% C.I.)	
	Inhale	Do Not Inhale
European Study [a]	28.1 (5.7-22.4)	5.2 (2.7-10.0)
ACS CPS II [b]	5.1 (4.0-6.6)	3.3 (2.3-4.7)

[a] Boffetta et al., J. Natl. Cancer Inst. 91:697-701 (1999)
[b] Shapiro et al., J. Natl. Cancer Inst. 92:333-337 (2000)
[c] Hecht and Hoffmann, J. Natl. Cancer Inst. 92:2040 (2000)

Metabolism of NNK to NNAL and NNAL-Glucs



S.E. Murphy and W. Kuehl, Proc. AACR 42:162 (2001)
P. Upadhyaya, E.J. McIntee, S.S. Hecht, Chem. Res. Toxicol 14:555 (2001)
Ky-Anh Le, S.G. Carmella, P. Upadhyaya, P. Villalta, S.S. Hecht, 2001

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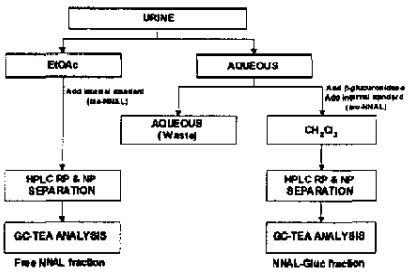
Source: https://www.industrydocuments.ucsf.edu/docs/sgmj0001

FLOW CHART OF NNAL ASSAY

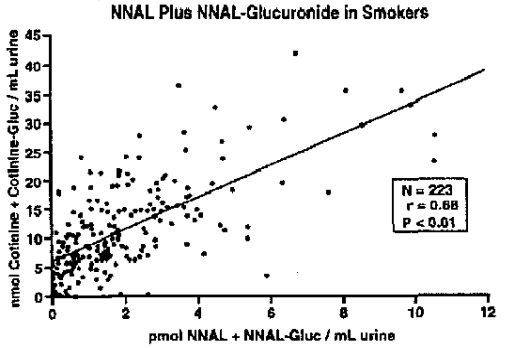


Applications of the NNAL Assay in Tobacco Users

- Estimation of NNK dose in smokers[1]
- Comparison of NNAL-Gluc/NNAL ratios[2]
- Ethnic differences in metabolism[3]
- Effects of diet and chemopreventive agents on NNK metabolism[4,5]
- Stereochemistry of NNAL and NNAL-Gluc[6]

[1]S.G. Carmella et al., Cancer Res., 53: 721 (1993); [2]S.G. Carmella et al. CEBP 4: 635 (1995); [3]J.P. Richie et al. CEBP 6: 783 (1997); [4]S.S. Hecht et al. CEBP 4: 877 (1995); [5]E. Tashi et al. CEBP 6: 517 (1997); [6]S.G. Carmella et al., Cancer Res., 59: 3602 (1999)

Correlation Between Cotinine Plus Cotinine-Glucuronide and NNAL Plus NNAL-Glucuronide in Smokers



S. S. Hecht, J. Natl. Cancer Inst. 91: 1194-1210 (1999)

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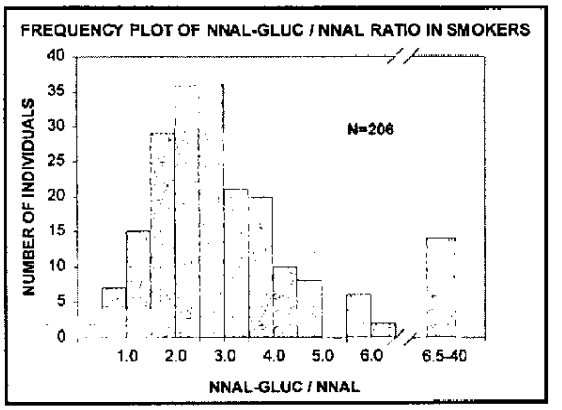
Source: https://www.industrydocuments.ucsf.edu/docs/sgmj0001



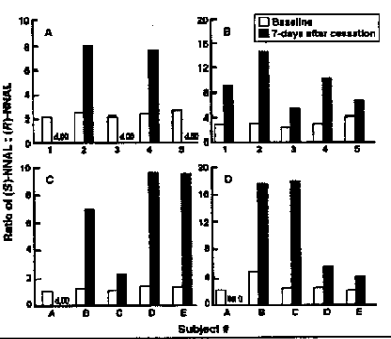
Pharmacokinetics of NNAL in Smokers and Snuff-Dippers

	Smokers	Snuff Dippers	P
$t_{1/2\alpha}$ (days)	3.35 ± 1.66 (n=22)	1.32 ± 0.85 (n=9)	0.004
$t_{1/2\beta}$ (days)	45.2 ± 26.9 (n=22)	26.3 ± 16.7 (n=10)	0.05

S.S. Hecht, S.G. Carmella, M. Chen, C. Zimmerman, J. Jensen, D. Hatsukami, et al., Cancer Res. 59:590-596 (1999)

S.S. Hecht, S.G. Carmella, M. Ye, K. Le, J. Jensen, C. Zimmerman and D. Hatsukami, Cancer Res. 61, in press (2001)

Ratio of (*S*):(*R*)-NNAL and NNAL-Gluc after Cessation of Tobacco Use



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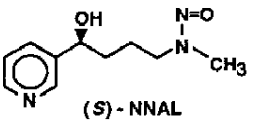
Source: https://www.industrydocuments.ucsf.edu/docs/sgmj0001



(*S*) - NNAL

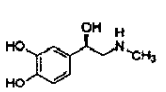
- (*S*)-NNAL is excreted more slowly than (*R*)-NNAL in humans, suggesting the presence of a receptor
- (*S*)-NNAL selectivity accumulates in rat lung and is excreted more slowly than (*R*)-NNAL
- (*S*)-NNAL is a more potent lung carcinogen than (*R*)-NNAL in A/J mice

P. Upadhyaya et al., Carcinogenesis 20:1577 (1999)
C. Zimmerman et al., Carcinogenesis 22: in press (2001)
S.S. Hecht et al., Cancer Res. 61: in press (2001)

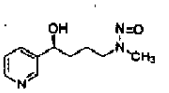
NNK is a ß-Adrenergic Receptor Agonist and Enhances Arachidonic Acid Synthesis in Human Lung Adenocarcinoma Cells

H. Schuller et al, Cancer Res. 59: 4510 (1999)

Structures of (*S*)-NNAL and (*R*)-epinephrine



(*R*)-epinephrine



(*S*)-NNAL

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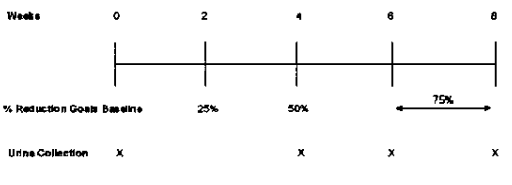
Source: https://www.industrydocuments.ucsf.edu/docs/sgmj0001

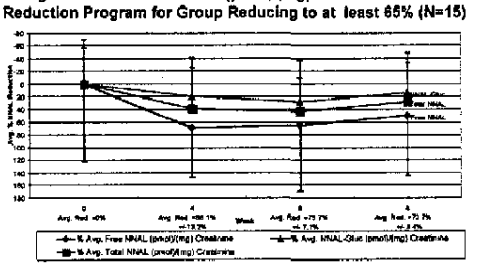
Smoking Reduction Study Design



Subject Inclusion Criteria
-Cigarette smokers between the ages of 18-70
-Interested in reducing but not quitting
-Smoking between 20-40 cigarettes daily

Results of Reduction Trial

Average % Reduction of NNAL (pmol)/(mg) Creatinine vs Week of Reduction Program for Group Reducing to at least 65% (N=15)



Applications of the NNAL Assay: Non-Smokers

- **Uptake of NNK by non-smokers exposed to ETS**
  - **Chamber study[1]**
  - **Occupational study[2]**
  - **Spouse study[3]**
  - **School children[4]**
- **Transplacental uptake of NNK[5,6]**

[1]S. S. Hecht et al., N. Engl. J. Med. 329: 1543-1546 (1993); [2]W. D. Parsons et al., Cancer Epidemiol. Biomarkers, Prev., 7: 257-260 (1998); [3]K. E. Anderson et al., J. Natl. Cancer Inst., 93: 378 (2001); [4]S.S. Hecht et al., CEBP 10 (2001); [5]G. M. Lackmann et al., J. Natl. Cancer Inst., 91 (1999); [6]A. Milunsky et al., Prenatal Diagnosis 20: 307 (2000)

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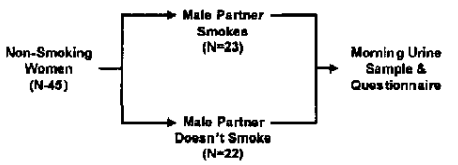
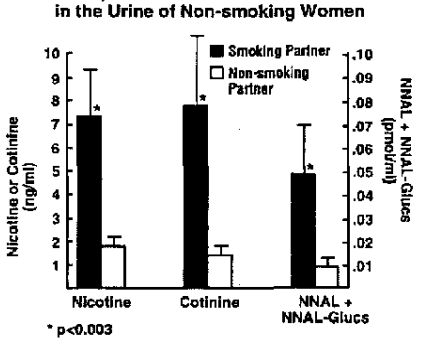
Source: https://www.industrydocuments.ucsf.edu/docs/sgmj0001

Design of Environmental Tobacco Smoke Study in Non-Smoking Women



Exclusions: Workplace or leisure ETS exposure

Nicotine, Cotinine and NNAL + NNAL-Gluc in the Urine of Non-smoking Women



K.E. Anderson et al. JNCI 93: 378 (2001)

Relative Risks for Lung Cancer and Relative Amounts of NNAL

	NNAL + NNAL-Gluc (pmol/ml)	% Excess Risk for Lung Cancer
ETS-Exposed Non-Smokers	0.05	20
Smokers	2.0	1400-1800
Relative %	2.5	1.1 – 1.4

W.J. Blot and J.K. McLaughlin, JNCI 90:1416 (1998)
S.S. Hecht et al., Cancer Res. 59:590 (1999)
S.S. Hecht et al., Cancer Epidemiol, Biomarkers & Prev., in press (2001)

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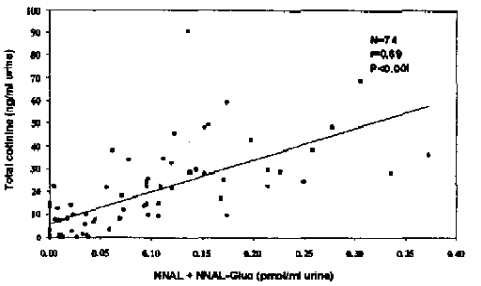
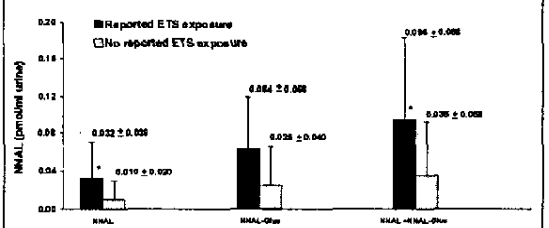
Source: https://www.industrydocuments.ucsf.edu/docs/sgmj0001

CORRELATION BETWEEN TOTAL URINARY COTININE AND TOTAL URINARY NNAL IN SCHOOL CHILDREN

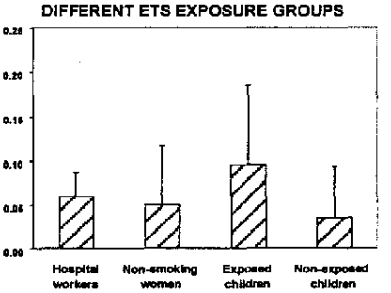


NNAL LEVELS IN CHILDREN WITH REPORTED AND NO REPORTED ETS EXPOSURE, ACCORDING TO QUESTIONNAIRE



S.S. Hecht et al., CEBP 10, in press (2001)

COMPARATIVE LEVELS OF TOTAL NNAL IN DIFFERENT ETS EXPOSURE GROUPS



ETS EXPOSURE GROUPS

S.S. Hecht et al., CEBP 10, in press (2001)

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Source: https://www.industrydocuments.ucsf.edu/docs/sgmj0001

Variation in NNK Uptake by Nonsmokers Exposed to ETS

Study	Fold variation[a]
Hospital workers	22
Spouses	31
Children	93

[a]. Detected values only

Specific Biomarkers of Carcinogen Uptake from ETS

Carcinogen in ETS	Biomarker	Effect of ETS exposure
Benzene	*trans, trans*-muconic acid in urine	Mixed results
PAH	1-hydroxypyrene in urine	None in most studies
	hydroxyphenanthrenes in urine	
	albumin adducts	
	hemoglobin adducts	
Aromatic amines	hemoglobin adducts	Mixed results
NNK/NNN	hemoglobin adducts	None
NNK	NNAL and NNAL-Gluc in urine	Consistently increased

Summary

- **Urinary biomarkers can be very useful in assessing tobacco carcinogen uptake.**
- ***Well-established biomarkers are available for* some of the important tobacco smoke carcinogens such as benzene, PAH, and NNK.**
- **Urinary NNAL is useful for understanding human NNK exposure, metabolism, and mechanisms.**
- **Urinary NNAL is an ideal biomarker for assessing relatively recent exposure to the lung carcinogen NNK from ETS.**

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Source: https://www.industrydocuments.ucsf.edu/docs/sgmj0001

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  - Menglan Chen
  - Ming Ye
  - Pramod Upadhyaya
  - Peter Villalta
- *Ken Sexton*
- Dorothy Hatsukami
- Cheryl Zimmerman
- Kristin Anderson

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Carcinogens I: Urinary Biomarkers of Carcinogen Uptake

Stephen Hecht, PhD

Notes:

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